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14. ABSTRACT

The purpose of this project was to develop the first multiuser micro and nanofabrication facility at The City College of New York (CCNY). Four pieces of equipment were acquired under this award: (i) Spin Coating System from Laurell Technologies; (ii) Ozone Stripper from Samco; (iii)Reactive Ion Etcher from Samco; and (iv) Evaporator/Sputtering system from Denton Vacuum. In addition, our school provided additional matching funds that enabled this research group to also purchase a Deep-UV Exposure System from Optical Analysis Instruments. This facility has fostered several research grant awards and applications from the PIs, the development of undergraduate and graduate courses involving micro and nanotechnology at CCNY, and enabled the PIs to mentor a total of 15 undergraduate and 11 graduate researchers via projects that utilize the facility.

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Development of a Micro- and Nano-fabrication Facility
FINAL REPORT

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Research Activities

Four pieces of equipment were acquired under this equipment award: (i) Spin Coating System from Laurell Technologies; (ii) Ozone Stripper from Samco; (iii)Reactive Ion Etcher from Samco; and (iv) Evaporator/Sputtering system from Denton Vacuum. In addition, our school provided additional matching funds that enabled this research group to also purchase a Deep-UV Exposure System from Optical Analysis Instruments. In total, the cost of the equipment for our facility was $244,025, of which the school contributed $44,025, exceeding the original promised matching of $20,000.

This equipment award from the Department of Defense (DoD) has allowed The City College of New York (CCNY) to build an operational micro and nanofabrication facility that has fostered many successful research grant applications from the PIs, development of undergraduate and graduate courses involving micro and nanotechnology at CCNY, and enabled the PIs to mentor a total of 15 undergraduate and 11 graduate researchers via projects utilizing the facility.

Outreach and Funding

The PIs have developed a concerted effort to recruit and mentor CCNY undergraduates from traditionally under-represented ethnic groups in various research projects that incorporate the micro/nanofacility at their core. Further, multiple undergraduates performed sponsored research during the summer months as a result of projects generated from the development of this facility. Our success in increasing graduate enrollment, particularly with a diverse background, depends in part on preparing a cadre of well-prepared and interested undergraduate students from our own campus. The acquired equipment has provided a significant educational impact on the CCNY student body by enabling the five co-PIs to upgrade and develop new courses that utilize the equipment and integrate research material into curricula in addition to their research efforts, as well as obtain funding from public and private agencies that hinged upon the PIs abilities to utilize the micro and nanofabrication facility. Professor Vazquez has developed one undergraduate course entitled, '*Microfabrication and Nanotechnology*,' as well as one graduate course, '*Microfluidics in Biotechnology*,' as a result of the acquired equipment. Prof. Gilchrist has initiated a new laboratory course titled '*Cell and Tissue Engineering*' which is

heavily populated by various engineers and scientists at the graduate and undergraduate level. Profs Vazquez and Gilchrist have further utilized the acquired equipment to obtain data that resulted in research awards from the National Science Foundation (NSF), the National Institutes of Health (NIH), and the Pediatric Brain Tumor Foundation (PBTF). These PIs have also been able to develop collaborations with researchers from the Hospital for Special Surgery (HSS) and Memorial Sloan Kettering Cancer Center (MSKCC) as a result of the newly developed fabrication center. Prof. Couzis and Maldarelli have created a series of graduate level courses related to interfacial phenomena, surface engineering and characterization at interfaces. These courses are open to upper level undergraduate students and rely heavily upon the equipment available. Lastly, Prof. Rumschitzki routinely teaches the graduate level transport and reaction engineering graduate courses. By enabling the development of these courses, the equipment acquisition will also inevitably insure that CCNY students are better prepared for industry and are aware of the various industrial opportunities available to them upon graduation.

Lastly, the educational impact of this facility can also be measured by the new pool of applicants of students and faculty alike. It is well documented that modern laboratories and facilities attract new, highly motivated and dedicated students at the undergraduate as well as graduate level. In line with this plan, we have acquired several students from interests in the projects which utilize the new equipment, including 8 students from Chemical Engineering and 4 from Biomedical Engineering.

Training

Six graduate students and two undergraduate have received training and have been using the new equipment. In addition two new graduate students are currently being trained on the machines. Training means that all students receive a short intensive course on the fundamentals of microfabrication and nanotechnology, and they familiarize themselves with the operating procedures of the particular piece of equipment.

RESEARCH PROJECTS

Below are descriptions of funded and submitted research projects that have been developed as a result of the CCNY Micro and Nanofabrication Facility. The PIs have additionally presented over 10 research papers and 15 seminars based upon these projects in the past year, at such venues as the annual meetings of the American Institute of Chemical Engineers (AIChE) and The Biomedical Engineering Society (BMES).

A. Fabrication of Nanoisland Patterns of Chemical Functionality and their Application for the Production of Nanocrystals and Biosensing (Couzis, Gilchrist, Maldarelli)

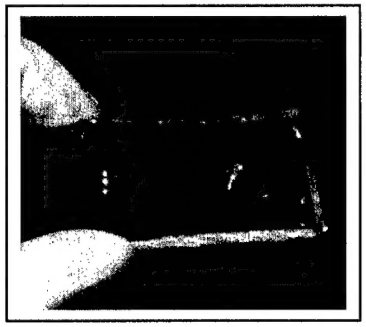
A.1. Unilamellar Vesicles: We are developing protocols to array individual, intact small unilamellar vesicles (liposomes) onto surfaces with potential applications as biosensor probes as well as for drug delivery. In the ongoing research, the substrates used are prepared by Micro-contact Printing (MCP) of islands of microscale and sub-microscale dimensions using silanes with amine (positively charged) terminal groups onto smooth silica substrates. Typically the silanes used in this step are 3-aminopropyltrimethoxy silane (APS) or p-aminophenyltrimethoxy silane (AphMS). The background phase is then put down using sequential adsorption of Polyethylene glycol (PEG) terminated silanes from solution using a proper solvent. From our previous research experience,

PEG terminated SAMs are inert to liposome adsorption and unraveling. Negatively charged liposomes of 1 micron diameter are prepared using low Tg formulations. The patterned SAM substrate is then exposed to the liposome solution, which results in adsorption of intact liposomes onto islands by electrostatic attraction. By situating the liposomes in the amine islands surrounded by the PEG terminated matrix, the liposomes are prevented from unraveling. We confirm the presence of intact arrayed liposome on these surfaces using Fluorescence microscopy, Confocal microscopy, AFM (Atomic Force Microscopy), FTIR (Fourier Transform Infrared) Spectroscopy, and Ellipsometry.

B. Surfaces for Molecular Recognition in Biosensing and Assaying (Couzis, Gilchrist, Maldarelli, Rumschitski, Vazquez)

B.1. Multivalent Nanoprobes to Target Intracellular Proteins (NSF-funded: Gilchrist and Vazquez)Measurement and regulation of the spatial and temporal organization of signaling complexes within a living cell have long been hailed as the key to regulating cell function and behavior. However, the possibility of tracking and controlling targeted proteins has only recently been enabled through the rapid advances of nanotechnology. The localization of proteins of interest has been traditionally accomplished via one to one monovalent binding with one fluorescently labeled molecule. However, recently developed nanoparticles offer greater signal intensity and duration, as well as the ability to bind multiple protein targets. These highly multivalent particles are efficiently transported into the cell at low concentrations, but their effects on targeted molecules have yet to be well established. It is likely that multivalent binding to target proteins engaged in signaling processes will generate measurable perturbations in cell behavior. This exploratory research proposes to develop quantitative measures of the changes in one cell function, chemotaxic migration, induced by the multivalent binding of one adapter protein needed to initiate chemotaxic signal cascades, Growth Factor Receptor Bound-2 (GRB2). Measurable changes in cell behavior will lead to the exciting possibility of using multivalent nanoprobes to alter or direct cell behavior as a new means for therapeutic intervention.

B.2. A Novel Nano-Microfluidic System for Intracellular Sensing (NSF-funded: Vazquez, Gilchrist) Sensing elements play pivotal roles in a wide variety of applications, including detection of biotoxins for food safety and homeland security. Current sensor development has been driven by objectives of reduced cost, diminished size, and superior accuracy as a result of the wide adaptation of microfluidic technologies and the soaring pace of nanotechnological innovations. The linkage between nanotechnology and microfluidics facilitates the development of cell-based biochemical sensor networks with improved sensitivity and robustness, as well as superior abilities to respond to minute changes within cellular systems. The quantization of intracellular distributions of target proteins in living cells provides a vital method for elucidating molecular details of cell signaling. This project's aim is to develop a cell-based, microfluidic sensor network that is able to utilize nanosensors to, both, detect changes in one targeted intracellular protein distribution, and exhibit multivalent binding to alter that protein's dynamics. It is our hypothesis that multivalent binding will affect cell behavior, directly, by depleting local concentrations of the targeted protein and affecting the protein-protein interactions required for



specific cell behavior. This project will specifically develop an original microfluidic network (the *μStar*) to study the effects of multivalent nanosensors on the fundamental cell process of chemotaxic migration. The system will enable single-cell imaging and quantitative analysis of nanosensor dynamics once inducted within viable fibroblast cells.

C. Imprinting Wetability Gradients Along Microchannel Walls for the Active Control of Droplet movement in Microfluidic Circuits (Couzis, Maldarelli)

C.1. Microscale total analysis systems are prototype devices consisting of a microfluidic network on a single chip which integrates, by means of fluid flow in microchannels, chemical reaction, separation and detection to undertake automated chemical and biochemical assays. In these prototypes, fluid is moved by means of pressure or electrokinetic forces powered by off-chip energy sources. In microfluidic systems, drops are a covenient method for undertaking assays as reagents and samples can easily be combined, and dropwise handling facilitates the sequential assaying of individual samples. The motions of drops in microfluidic channels are dominated by capillary forces, and methods which take advantage of surface forces to actuate fluid motion – such as thermocapillarity and electrowetting – require less energy and can be empowered by energy sources on the chip, providing the additional advantage of portability as well as small size.

In this work, we develop a capillary actuated, self propelling method to move aqueous drops in a microchannel which is based on inscribing, on the inside surface of the channel, a gradient in the surface energy of the channel wall (against air) which increases along the axial direction of the channel. The motion of the drops due to surface chemical gradients on flat surfaces was first reported by Manoj K. Chaudhary and George K. Whitesides (Science 1992). The advantage in going to a cylindrical geometry is that the drop experiences more force in the axial direction as the contact area (with the surface) of the drop is more in case of a capillary tube than a flat surface. When a liquid drop is situated along the gradient, the aqueous/solid interfacial energy is smaller, and therefore the aqueous phase contact angle lower, at the end of the drop in contact with the larger air/solid surface energy, then it is at the opposite end of the drop. This difference in contact angles propels the aqueous phase in the direction of increasing wettability. In our application, gradients in air/solid energy are imprinted on the inside surface of borosilicate glass tubes by adsorbing self assembled monolayers (SAMs) of a fluoro-terminated silane (heptadecafluoro-1,1,2,2-tetra-hydrodectrichtorosilane) from a chloroform depositing solution. The adsorption renders the surface hydrophobic due to the terminal fluoro groups of the assembling silane monolayer; the larger the contact time of the solution with the surface, the greater the density of adsorption and the lower the surface energy of the glass surface against air. The gradient is formed by forcing an advancing slug of the depositing solution through the capillary tube allowing the end of the tube in longer contact with the depositing solution to have the lower surface energy

D. Separation of Biomolecules within Microchannels and Nanolabeling of Proteins during Cell Migration (Vazquez)

D.1. A Microfluidic System to Co-Localize ERK Proteins During Medulloblastoma Dispersal (PBTF: Vazquez) This project will develop a novel, nano-microfluidic system to examine the mechanisms of medulloblastoma dispersion via visualization of cascade proteins during real time migration. This research will be the first to facilitate measurements of the spatial and temporal activity of growth factor receptors (PDGFR) and Extracellular Signal Regulated Kinase (ERK) cascade proteins during real-time medulloblastoma migration. Intracellular nanoprobes will be used to track and detect

translocations of intracellular Growth Factor Receptor Bound 2 (GRB2) and Son of Sevenless (SOS) proteins in order to determine how binding with PDGFR stimulates the translocation of signaling proteins during initial and steady state cell motility. This novel system uniquely integrates frontiers in biology and engineering to perform translational experiments that elucidate unprecedented mechanistic details of brain tumor dispersal. Results of the project will facilitate future research translating this nanotechnology into *in vivo* models for development of therapies that reduce medulloblastoma dispersion via multivalent nano-binding to surface proteins and downstream signaling molecules.